REMARKS

Claims 1, 2, 6 to 13, 17 and 21 to 30 are pending in this application. Claims 8, 12, 17, 23, 24, 28, and 30 were amended. Entry and allowance of the pending claims is requested in view of these remarks, which address issues raised by the Examiner, Dr. Kerr, in the order she presented them in the above-mentioned Action, with cross-referencing to her numbered sections.

This invention relates to the methods of promoting central nervous system axon growth by administration to a patient in need of axon regeneration a composition containing an effective amount of at least one rho protein inhibitor in amounts effective to inhibit rho or rac and stimulate neurite outgrowth.

Drawings. 3. Applicant submits herewith replacement drawing sheets 2 (Figures 2A and 2B), 3 (Figures 2C and 2D), 4 (Figures 3A-3C), 5 (Figures 4A-4C), 6 (Figures 5A and 5B), 6 (5A and 5B), 7 (Figures 6A and 6C), 8 (Figures 7 and 8) which provide the changes suggested by the Notice of Draftperson's Patent Drawing Review (PTO Form 948) dated 29 June 2001.

Claim Rejections Maintained Under 35 U.S.C. § 112. 10. Claims 1, 2, 6 and 9 to 12 were rejected as indefinite under the statute for reciting the term "rho protein inhibitor". The rejection is respectfully traversed. The claim was amended after the last Office Action by the addition of functional language --in amounts effective to inhibit rho or rac and stimulate neurite outgrowth-- to particularly point out the preferred embodiment of the invention. As amended, the claim was circumscribed to encompass rho inhibitors that exhibit this functionality, so that the claim defines what is encompassed by the invention. Rho proteins are a known subfamily of GTPases of which rho and rac are closely related members. Rho protein inhibitors useful in methods of the invention are those that inhibit rho or rac and stimulate neurite outgrowth. The group includes previously described inhibitors as well as those which can be readily identified using screens described in the specification on page 12 and in the literature.

As Dr. Strittmatter explains in paragraphs 3 to 5 of a 37 C.F.R. § 1.132 Declaration accompanying this response, rho protein GTPases are a relatively small group of proteins that have been studied in considerable detail. Moreover, a number of rho protein inhibitors are known in addition to those exhibiting *C. botulinum* C3 exoenzyme activity illustrated in the examples, so that investigators in the field use the term to functionally delineate a group comprising a variety of compounds that inhibit rho proteins in different systems. Rho protein inhibition observed using newly tested compounds is often compared to the irreversible inhibition observed when *C. botulinum* C3 exoenzyme is incubated with a rho protein in publications describing new inhibitors. Indeed, the fact that the group of rho protein inhibitors is well known and studied by different groups of investigators underscores the patentability of the invention. Though they have been studied for some time, Applicant is the first to find that rho protein inhibitors can stimulate neurite outgrowth, thus promoting axon regeneration.

Accompanying the Declaration are the full texts of several recent papers Dr. Strittmatter summarizes in paragraphs 4 and 9 of the Declaration. All of these refer to an earlier literature describing rho proteins and their properties, thereby supporting Applicant's position that rho protein inhibitors are well defined in the art. Maddala, *et al.*, for example, reported inhibition of rho and rac GTPases by lovastatin and C3 exoenzyme in a human lens epithelial cell line (*Invest. Ophthalmol. Vis. Sci. 42*: 2610-26157, 2001). Ohnaka, *et al.*, observed rho inhibition by pitavastatin in cultured human osteoblasts (*Biochem. Biiophys. Res. Commun. 287*: 337-342, 2001). Smirnova, *et al.*, observed the promotion of neurite outgrowth by lovastatin in two cell line cultures (*J. Neurobiol. 48*: 87-100, 2001). In the paper, neurite outgrowth using lovastatin inhibition was similar to what was observed using a highly cell-penetrant chimeric molecule consisting of C3 exoenzyme from *C. botulinum* and diphtheria toxin.

Takemoto, *et al.*, reported rho inhibition in the presence of simvastatin and a geranylgeranyltransferase (abbreviated GTI or GGTI) inhibitor denoted GTI-286, and compared inhibition with *C. botulinum* C3 exoenzyme *in vitro* in cultured cardiac

myocytes and *in vivo* in rat hearts (*J. Clin. Invest. 108*: 1429-1437, 2001). Nègre-Aminou, *et al.*, also described rho inhibition by simvastatin, but in a smooth muscle model (Nègre-Aminou, P., *et al., Biochem. Pharmacol. 61*: 991-998, 2001). Eberlein, *et al.*, reported inhibition of rho proteins using lovastatin, simvastatin, and GTI-286 in cultured human renal fibroblasts (*Brit. J. Pharmacol. 133*: 1172-1180, 2001). Lesh, *et al.*, reported inhibition of rho proteins in cultured human trachea smooth muscle tissue in the presence of GGTI-286 and another potent, highly specific inhibitor denoted GTTI-2147 (*Am. J. Physiol. Lung Cell Mol. Physiol. 281*: L824-L8315, 2001). Adnane, *et al.*, described rho inhibition by yet another inhibitor denoted GGTI-298 in a human pancreatic carcinoma cell line (*Mol. Cell Biol. 18*: 6982-69701, 1998).

Cohen, *et al.*, observed rho inhibition in cultured human smooth muscle mammary artery cells by a number of compounds depicted as primarily isoprenyl analogues (*Biochem. Pharmacol. 61*: 1061-1068, 2000). Sasaki and Takai have described rho inhibition by small soluble proteins called Rho GDIs (*Biochem. Biophys. Res. Com. 245*: 641-645, 1998). Linseman, *et al.*, have published studies showing *Clostridium difficile* toxin B inhibits several members of the rho family (*J. Biol. Chem. 276*: 5622-5628, 2001). Västrik, *et al.*, have recently described cell permeant peptide inhibitors of rac and their ability to block the action of certain axon repulsive agents (*Curr. Biol. 9*: 991-998, 1998).

Read against the considerable rho protein literature, Applicant respectfully submits that the term "rho protein inhibitor" is not an indefinite term, particularly when coupled with certain functional properties that delimit the class, *i.e.*, inhibitors that inhibit rho or rac and stimulate neurite outgrowth. Applicant and other investigators before him have thus defined many assays that can be used to detect and measure rho protein inhibition and commercially available reagents that can be used in the assays.

Applicant anticipates that numerous compounds that stimulate neurite outgrowth will now be identified using screens of the type described in the specification on page 12

at lines 5 to 17 to test thousands of compounds. Applicant provides instruction to skilled workers to accomplish this identification, and should not be limited to claims to only a couple of inhibitors illustrated in the Examples. Indeed, as described in his Declaration, Applicant has observed axon regeneration *in vitro* and *in vivo* using another previously described rho protein inhibitor denoted Y-27632, a synthetic carboxamide derivative recently reviewed by Narumiya, S., *et al.* (*Methods Enzymol. 325*: 273-284, 2000). His experiments are summarized in paragraphs 6 to 8 and Figures 1 to 3 of his Declaration. Neurite outgrowth was stimulated in E13 DRG neuron cultures treated with CNS myelin axon inhibitors, in whole CNS myelin preparations treated with CNS myelin axon inhibitors, and after spinal injury in rats. C3 exoenzyme had the same effect. The results are graphically compared in Declaration Figure 1. The rho protein inhibitor Y-27632 clearly promoted neurite outgrowth, promoted axon regeneration, and functional recovery after spinal injury in the experiments.

Applicant has found a new use for previously described rho protein inhibitors, and a way to readily identify others useful in the practice of the invention by combining the teachings of his disclosure with information provided by the rho protein literature, particularly the use of statins and other compounds in addition to C3 exoenzyme as rho protein inhibitors. Applicant therefore respectfully requests that the objection to his language describing his pioneer invention be withdrawn, and that claims using the term be allowed.

11. Claims 8 and 17 were rejected as being indefinite for the term "C2/C3 inhibitor". The claims were amended in response to the rejection and at the suggestion of the Examiner, by adding a limitation particularly pointing out that the construct exhibits C3 exoenzyme activity, *i.e.*, that the construct ADP-ribosylates rho specifically and inactivates the G protein. Support for the amendment may be found in the specification on page 4 at lines 8 to 9. Claim 8 was further omitted by adding the word --exoenzyme-- after "construct" in line 2, to clarify the claim and have its language track the language of claim 8. Support for that amendment can be found in the specification on page 11 at line 14.

12. The rejection of claims 1, 2, and 6 to 11 under 35 U.S.C. § 112 was maintained as the claims require *in vivo* results for enablement (Office Action, page 6, lines 5 to 6), and the *in vitro* models in the specification together with the *in vivo* results presented in a recent paper by Lehmann, M., *et al.* (*J. Neurosci 19*: 7537-7547, 1999) were insufficient to support enablement (Office Action, page 6, lines 4 to 8). The rejection is respectfully traversed. As Dr. Strittmatter points out in his Declaration in paragraph 3, investigators in the field routinely employ *in vitro* models in the initial phases of neuronal research, as these are often predictive of *in vivo* results and are more convenient and economical than *in vivo* experiments at the outset of research on a given system. The properties of rho proteins and their inhibitors have not been observed to be different *in vitro* and *in vivo* in literature reports, and the results of Applicant's *in vitro* experiments have been confirmed *in vivo*.

The Examiner used language from a Bartsch, *et al.*, paper (page 1379, left column of *Neuron 15*: 1375-1381, 1995) to support the proposition that *in vitro* results are not predictive of *in vivo* results in CNS research. Bartsch, *et al.*, observed that myelin-associated glycoprotein (abbreviated MAG) did not appear to be involved in inhibiting axonal regeneration in MAG-deficient mice, though MAG seemed to be a neurite outgrowth inhibitor in *in vitro* experiments (Summary, page 1375, column 1, and column 2, first full paragraph). But the investigators do not state that MAG is the sole molecule hypothesized to contribute to the inhibitory activity of CNS glial cells or CNS myelin. On the contrary, several constituents of the extracellular matrix are suggested (column 2, lines 8 to 10). A monoclonal antibody denoted MAb IN-1 raised against neurite growth inhibitors neutralized part of the inhibition both *in vitro* and *in vivo*. The inhibitory effects of MAG were reported to be apparent only when the purified molecule was used as a substrate (page 1379, column 1, paragraph 2, lines 7 to 10). As stated by the authors (page 1379, column 1, paragraph 2, lines 1 to 3), the finding that MAG didn't appear to play a role in inhibiting neurite outgrowth in MAG-deficient mice simply argued against MAG as having a major role in inhibition. The possibility that MAG may have some role in normal mammals was not discounted, and a reading of the reference as

a whole underscores the fact that typical research protocols in the neurosciences involve initial *in vitro* experiments followed by *in vivo* work. If *in vitro* experiments were not predictive of *in vivo* results, scientists wouldn't bother with *in vitro* work. More important and telling, the Bartsch, *et al.*, paper's conclusions about MAG are not corroborated by other investigators, who have reported that MAG inhibits axon growth both *in vitro* and *in vivo* (Lehmann, *et al.*, cited above, column 1, lines 4 to 7, referencing 4 papers not mentioned by Bartsch, *et al.*).

As Dr. Strittmatter states in item 3 of his Declaration, published results of *in vivo* experiments with rho protein inhibitors confirm *in vitro* findings. Since *C. botulinum* C3 exoenzyme is well known as an irreversible rho protein inhibitor, it is often used as a standard for comparison purposes. Some papers publish *in vitro* results back to back with *in vivo* results; see, for example, Takemoto, *et al.*, cited above. More significant are the results using Y-27632 as a rho inhibitor to promote axon regeneration that Dr. Strittmatter reported in his Declaration in items 6 to 8 and the Figures. Inhibition was observed both *in vitro* and *in vivo*, and the results were similar to what was observed using the C3 exoenzyme.

Furthermore, the *in vitro* results with C3 exoenzyme reported in the specification Examples have been confirmed *in vivo* by another neurologist. Dr. Bernhard Mueller performed some *in vivo* experiments using a C2/C3 construct that exhibited C3 exoenzyme activity to stimulate nerve growth and restore motor function to animals with a spinal cord lesion. His results are summarized in a 37 C.F.R. § 1.132 Declaration accompanying this response. The construct he employed as a rho protein inhibitor was a chimeric fusion protein composed of C3-transferase (from *Clostridium limosum*) and a portion of the C2-toxin denoted C2I that he denoted C3-C2IN (Mueller Declaration, paragraph 5). Improvement sensor and motor function was observed after the spinal cords of rats were severed in animals treated with C3-C2IN that was not observed in untreated controls in two independent experiments (Mueller Declaration, paragraphs 9 to 11). Immunohistological examination showed neurite outgrowth stimulation by admin-

stration of the inhibitor (Mueller Declaration, paragraphs 12 to 18) Dr. Mueller concluded that rho protein inhibitors such as C3 exoenzyme, when administered *in vivo*, can stimulate axon regeneration and restore motor function (Mueller Declaration, paragraph 20). The *in vivo* experiments with two rho protein inhibitors reported in the two Declarations fully support Applicant's claims to the promotion of both sensor and motor axon regeneration using the inhibitors.

The group of investigators who authored the Lehmann, *et al.*, paper cited above have recently reported axon regeneration and functional recovery after spinal cord injury in adult mice treated with C3 exoenzyme. This research is summarized in two abstracts accompanying this amendment. The Lehmann, *et al.*, paper reported that C3 exoenzyme treatment of lesions induced by crushing optic nerves of adult rats resulted in axon regeneration. The group then went on to study the effects of C3 exoenzyme on spinal nerve injuries. McKerracher, *et al.*, *Soc. Neurosci. Abst. 26*: Abst. No. 230.4 (2000) reported "robust" axon regeneration in transected spinal cords of adult mice treated with C3 exoenzyme. Then McKerracher, L., *et al., Soc. Neurosci. Abst. 27*: 2120 (2001), obtained "remarkable" recovery 24 hours after C3 exoenzyme treatment following a dorsal hemisection of mice spinal cords, and recovery continued over the next months. Axon regeneration was observed as well as functional recovery. Untreated animals showed no changes.

With regard to the claimed methods of treating specific injuries, Applicant traverses the conclusion (set out in the first paragraph on page 7 of the Office Action) that data does not support the methods. Both Dr. Strittmatter and Dr. Mueller delivered rho protein inhibitors to the site of spinal injury using perfusions of the lesions (Declaration paragraphs 7 and 6, respectively). The experiments support the mechanical introduction administrative route particularly pointed out in claim 2, and the acute spinal cord injury limitation set out in claim 9. Acute or chronic spinal cord injury, white matter stroke, and traumatic brain injury particularly pointed out in claims 9 to 10 are all pathological conditions involving axon death. Any of Applicant's claimed methods that involves the stimulation of axon regeneration would treat these conditions. Applicant

therefore believes that the claims are allowable and requests reconsideration of the rejection on this basis.

13. As summarized above, the rho protein literature provides a number of functional rho inhibitors including C3 exoenzyme and constructs and isolates exhibiting C3 exoenzyme activity derived from *C. botulinum, C. difficile, and C. limosum*; statins, *e.g.* lovastatin, simvastatin, and pitavastatin; Rho GDIs; some peptides; some geranyl-geranyltransferase inhibitors; some isoprenyl analogues; and Y-27632. Applicant's findings in his Y-27632 experiments reported in the Strittmatter Declaration provide *in vitro* and *in vivo* results that support the claims. The Lehmann, *et al.*, paper's *in vivo* results using C3 exoenzyme to promote axon regeneration in optic nerves support the claims, as do subsequent results from the same group in the treatment of spinal cord injuries with C3 exoenzyme reported in the McKerracher, *et al.*, abstracts. Dr. Mueller's results in his Declaration provide *in vivo* confirmation of the C3 exoenzyme *in vitro* experiments reported in the specification Examples also support the claims. Applicant therefore requests reconsideration of the rejection and allowance of the claims.

Claim Rejections Maintained Under 35 U.S.C. § 102(b). 15. Claim 13 was rejected as anticipated by Morii, *et al.* (*J. Biochem. 107*: 769-775, 1990). The claim was amended to correct this oversight in the preparation of the last Office Action response, converting it from a composition claim to a method claim in response to the rejection and tracking the language of claim 12, the method claim from which claim 13 depends. As amended, the claim should be allowable because Morii, *et al.* do not anticipate Applicant's method of stimulating axon regeneration using rho protein inhibitors and parent claim 12 was deemed allowable in item 14 of the Office Action.

Claim Objections. 17. Claim 12 was objected to for a computer glitch that printed the line number "5" over the "i" in "in" so that the first word on line 5 read "5n". The claim was amended to correct this double printing error. Claim 17 was

objected to for the misspelling of --*botulinum*--. The claim was amended to correct this typographical error. The undersigned thanks the Examiner for pointing these out so that they could be corrected.

New Claim Rejection Under 35 U.S.C. § 112. 18. Claim 8 was rejected under the statute as depending upon claim 24, which depends upon itself. In response to the rejection, the claims were amended to correct this typographical errors and clear up the confusion they elicited.

19. Claims 12, 13, and 25-27 were rejected as indefinite in the recitations of "rac protein" and "rho protein" in a Markush group of inhibitors in claim 12. The claim was amended to clarify that inhibitors are proper members of the group, and not the proteins themselves.

20. Claims 21-23 and 25-27 were rejected under 35 U.S.C. § 112 as indefinite in the recitation of "rho protein inhibitor". The rejection is respectfully traversed for reasons already discussed, namely, that rho protein inhibitors are known and described in the literature and assays for determining the functional activity of useful inhibitors of the invention are provided by the specification and by the scientific literature.

21. Claims 23 and 28 were rejected as confusing in the diction used to describe the C3 exoenzyme rho protein inhibitor. The claims were amended to standardize the language in all the claims, so that the inhibitor is described as an exoenzyme.

22. Claim 30 was rejected as indefinite in describing the C2/C3 construct. The claim was amended to track the language of amended claims 8 and 17 to overcome the rejection.

Applicant has made a significant advance for the promotion of axon regeneration in the treatment of patients in need of CNS recovery after acute or chronic spinal cord injury, white matter stroke, and traumatic brain injury. Reconsideration and allowance of

the amended claims is therefore respectfully requested. If the undersigned can advance the prosecution of this application in any way whatsoever, please call at the number listed below.

Respectfully submitted,

Mary M. Krinsky

MARY M. KRINSKY, Reg. No. 32423
79 Trumbull Street
New Haven, Connecticut 06511-3708
(203) 773-9544

Marked Up Version of Amendments Required by 37 C.F.R. § 1.121

8 (Amended). A method according to claim [24] 1 wherein the rho protein inhibitor is a chimeric *C. botulinum* C2/C3 exoenzyme construct having the actin ADP-ribosylation activity deleted from the C2 toxin and the C3 enzyme activity substituted therefor, so that the construct ADP-ribosylates rho specifically and inactivates the G protein.

12 (Amended). A method for promoting central nervous axon growth in a patient in need of axon regeneration by administering to the patient a pharmaceutical composition containing at least one rho protein inhibitor selected from the group consisting of a rac protein inhibitor, a rho protein inhibitor, a protein that inhibits both a rac protein and a rho protein, and mixtures thereof, [5n] in amounts effective to inhibit rho or rac such that neurite outgrowth is stimulated.

13 (Amended). A [composition] method according to claim 12 which comprises *C. botulinum* C3 exoenzyme.

17 (Amended). A method according to claim 12 wherein the composition comprises a chimeric C2/C3 *C. [botulimun] botulinum* exoenzyme contruct having the actin ADP-ribosylation activity deleted from the C2 toxin and the C3 enzyme activity substituted therefor, so that the construct ADP-ribosylates rho specifically and inactivates the G protein.

23 (Amended). A method according to claim 1 wherein the inhibitor is [a] *C. botulinum* C3 exoenzyme.

24 (Amended). A method according to claim [24] 12 wherein the inhibitor is *C. botulinum* C3 exoenzyme.

28 (Amended). A method for promoting central nervous system axon growth in a patient in need of axon regeneration comprising administering to the patient an effective amount of [a] *C. botulinum* C3 [inhibitor] exoenzyme.

30 (Amended). A method according to claim 28 wherein the composition comprises a chimeric C2/C3 *C. botulimun* exoenzyme contruct having the actin ADP-ribosylation activity deleted from the C2 toxin and the C3 enzyme activity substituted therefor, so that the construct ADP-ribosylates rho specifically and inactivates the G protein.